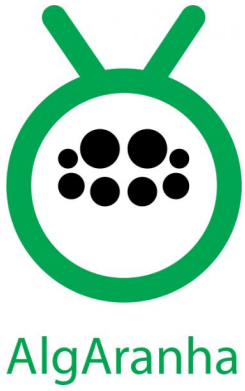
Assembly by restriction enzyme of Lh Masp 1 Type 2



Summary

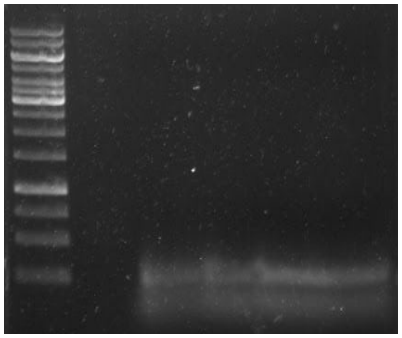
Another approach to address polymerization of spider silk monomers was performed through restriction enzyme cloning, in spite of additional scars that this technique.

----------09/02----------

MaSp1 type2 PCR product gel purification

Mireia

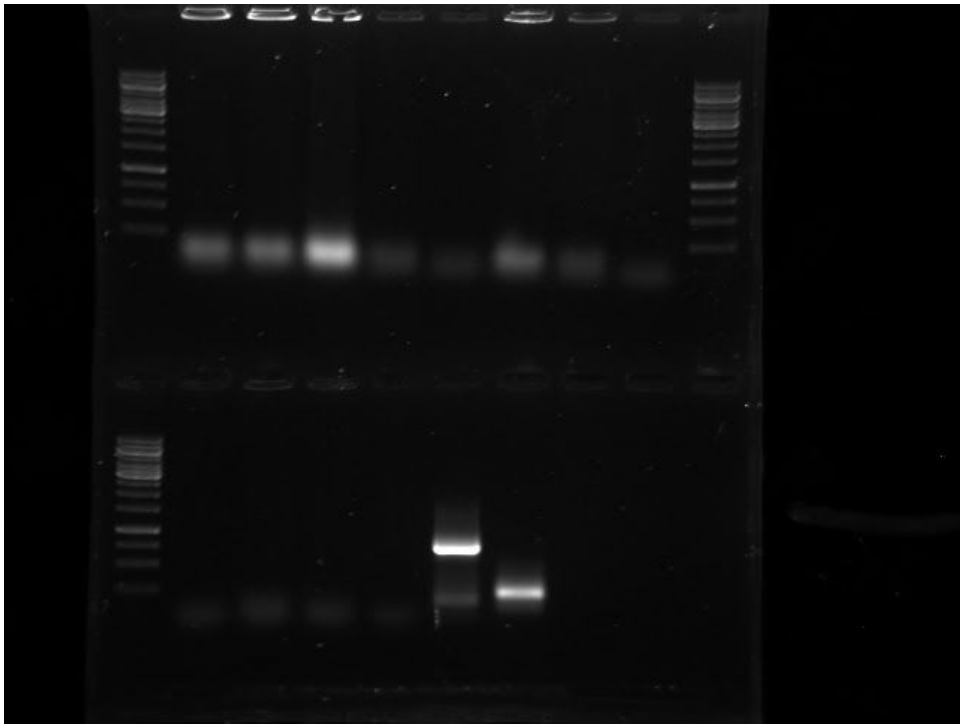
350 uL of MaSp1 type 2 PCR product were run on a 0.8% agarose gel (80 V, ~45 min)

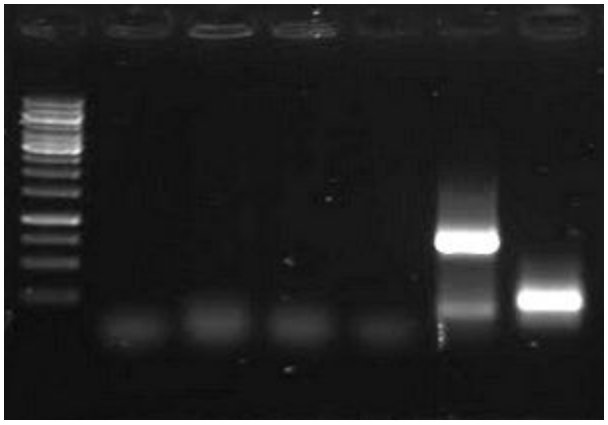


----------09/03----------

Mireia

MaSp1 type2 PCR to add overhangs





----------09/04 - 09/09 ----------

Mireia

MaSp1 type2 restriction cloning into pJP22

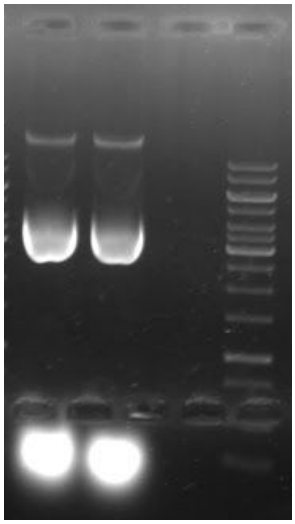
Mireia

Transformation of DH5α with pJP22-MaSp1-2X

----------09/10----------

Alkaline lysis of DH5α transformed with pJP22-MaSp1-2X

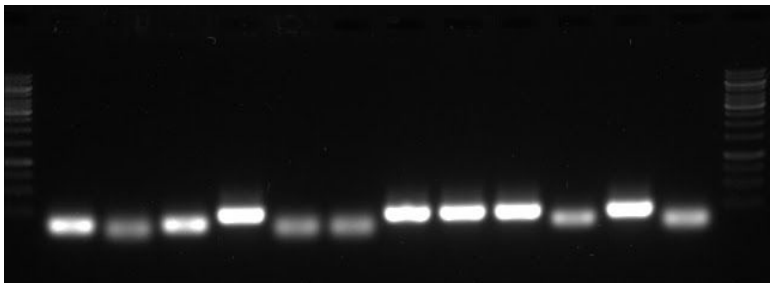
Mireia



----------09/13----------

Alkaline lysis PCR of DH5α pJP22-MaSp1-2X

Mireia



----------09/14----------

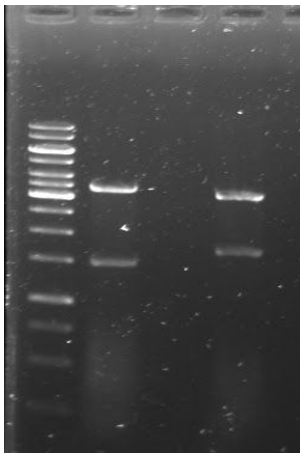
Digestion of pJP22-MaSp1-2X with BamHI and ScaI (A) / BglII and ScaI (B)

Mireia

A: **3531** bp + 1570 bp

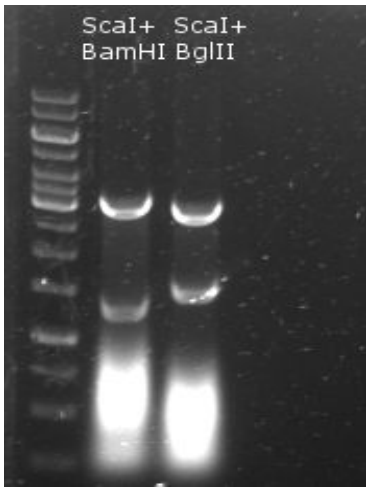
B: 3333 bp + **1768** bp

Bold number fragments contain a copy of the MaSp1 insert and are to be ligated to duplicate that insert and regenerate the Amp resistance gene.



Mireia and Felipe

As the PRC done on 9/19 showed the previous ligation wasn't successful, we decided to digest the 2X plasmid once again to try another ligation later. Digestion electrophoresis in agarose gel showed bands in the right spots:



OBS: band curviness due to putting too much DNA in the wells, what makes middle DNA travel faster through gel. Ends of the bands travel correctly and should be used for reference.

Ligation of pJP22-MaSp1-2X fragments A and B (2nd time)

Mireia and Felipe

Item	Volume
H_2O	1 uL
A (55,5ng/uL)	4 uL (~222 ng)
B (43ng/uL)	3 uL (~132 ng)
T4 Buffer 10X	1 uL
Ligase	1 uL
Total	10 uL

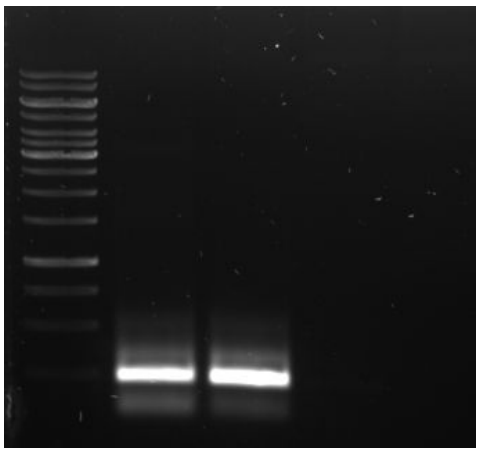
Reaction conditions:

	TEMP	TIME
30X	22°C	3 min
	37°C	3 min
	4°C	hold

----------09/21----------

PCR of MaSp1 4-mer ligation (2nd time)

Mireia



Result: 2-mer amplicon band.

Hypothesis: The primers can attach at the ends of the 4mer but also at the center, so probably we are obtaining only the 2mer amplicon. All primers attach and the polymerase is not able to amplify through the whole 4mer, because it finds an attached primer on its way.

So: transform and check for colony growth, miniprep and digest for 4mer confirmation.

Transformation of DH10B with the 2nd 4-mer ligation

Mireia

Electroporation of 50 uL DH10B with 2 uL ligation. (1 mm cuvette, 1800 V)

Result: 5 colonies grew on the LBCb plate.

----------09/22----------

Alkaline lysis of 5 MaSp1t2 4mer colonies

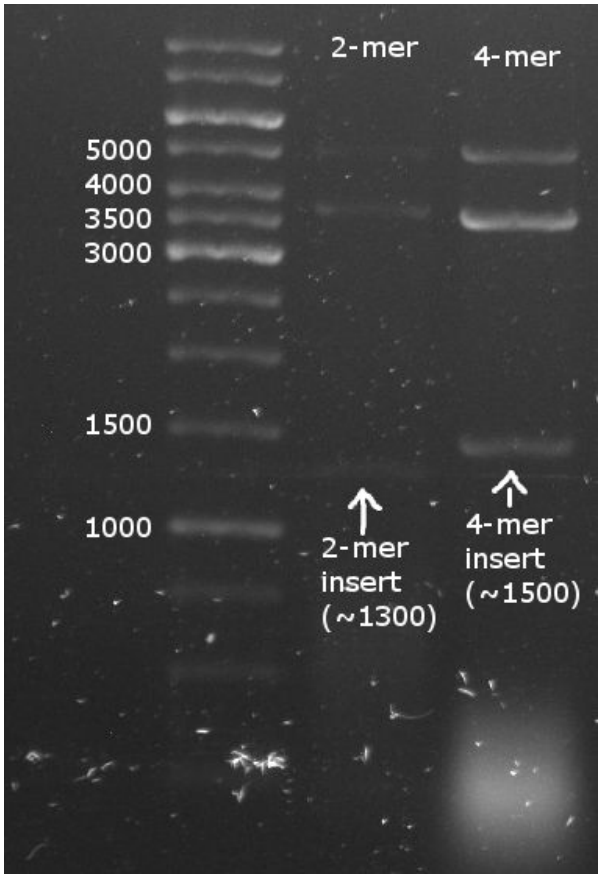
Mireia

Digestion of pJP22 MaSp1t2 4mer with PstI and KpnI for 4mer confirmation

Felipe and Mireia

As we couldn't see the insert bands due to RNA on the XhoI/BamHI digestion, we decided to try again with PstI/KpnI because that would form more discernable bands, at ~1300 (2mer) and ~1500 (4mer)

Item	Volume for 1 reaction	Volume for 7 reactions
PstI NdeI	1uL	7 uL(used 5)
KpnI	1uL	7 uL(used 5)
H_2O	11uL	77 uL(used 77+4)
Buffer	2uL	14 uL
Total	15uL	105 uL



Result: A ~1500 band showed we have the 4mer insert.

Digestion of pJP22-MaSp1-4X with BamHI and ScaI (A) / BglII and ScaI (B)

Mireia

A: BamHI + ScaI (Fast Digest Thermo) 1h ~37ºC

B: BglII + ScaI (Pharmacia) overnight ~37ºC

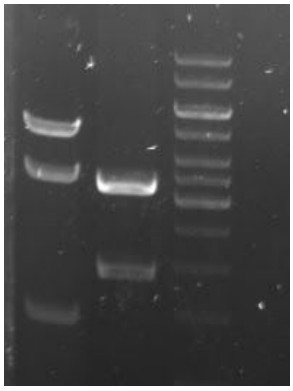
Result:

A: **3759** and 1570 (Not completely cut fter 1h at ~37ºC, after the gel it was left for 30 min at 37ºC)

B: 3333 and **1966**

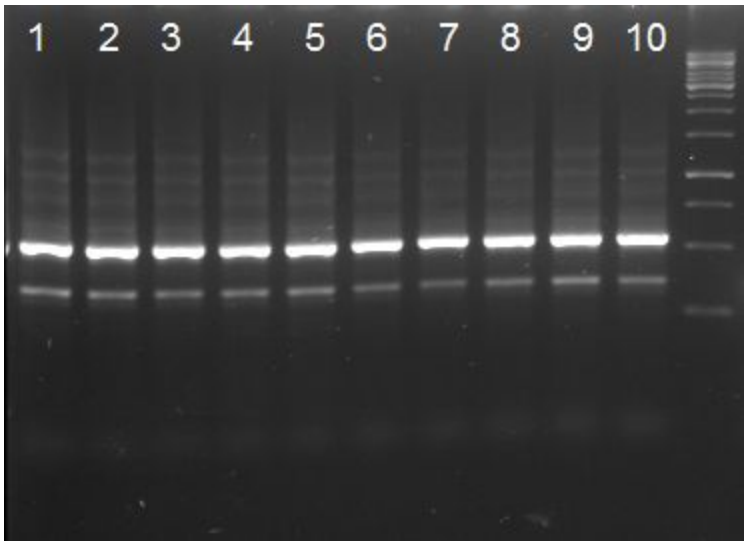
bold = contains the 4X MaSp1t2 insert.

5 uL restriction check. 45 uL were run afterwards to purify the bold fragments:



Ligation of pJP22-MaSp1-4X fragments A and B

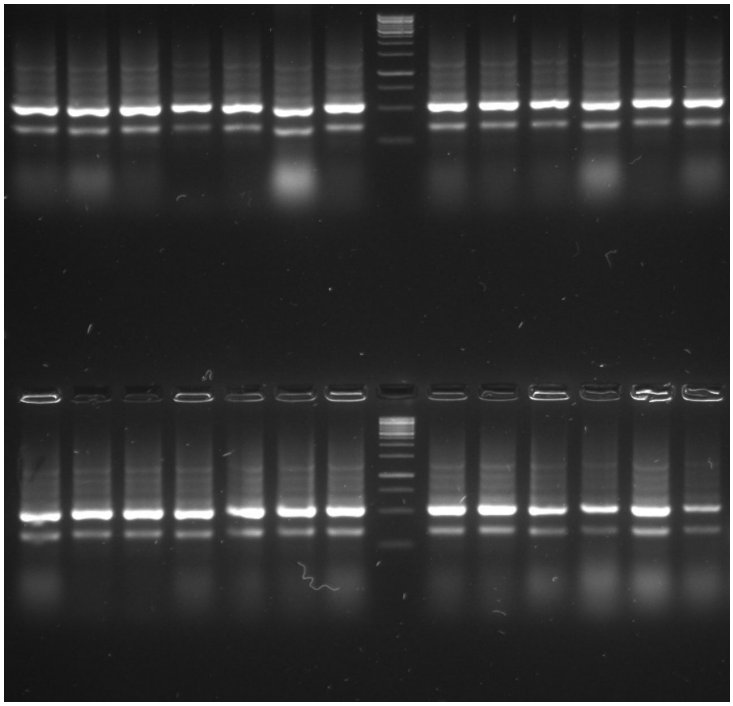
Colony PCR of the pJP22-MaSp1 8X transformation
Mireia



No 8mer band (~950b) was obtained.

Colony PCR 2 of the pJP22-MaSp1 8X transformation
Mireia

PCR done with more colonies this time (26) to confirm 8mer plasmid absence



No 8mer band (~950b) was obtained.

Digestion of pJP22-MaSp1-4X with BamHI and ScaI (A) / BglII and ScaI (B) (2nd time)
Mireia

Ligation of pJP22-MaSp1-4X fragments A and B (2nd time)